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Review article

Microalgae for biobutanol production – Technology evaluation and value proposition

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ABSTRACT

The depletion of petroleum and fossil fuels and the escalating problem of climate change motivate and compel an ongoing effort focusing on the development of renewable energy in the form of biofuels. Biobutanol is one such potent biofuel, attributing to similar characteristics as of gasoline, which manifests in easier public distribution based on the current oil and gas infrastructure. Also, the development of the third-generation biofuels sourced from cultivation of microalgae seems an outright promising prospect for renewable energy sources. This is mainly because of its inherent advantages in comparison to the previous methods of biofuel production from crops and plant waste. However, in spite of the ongoing efforts, the research targeting towards biobutanol production utilizing microalgal resources is insufficient. Working on the strengths of both may provide the much needed boost for the thriving biofuel industry, ultimately aiding to cope with global energy demand and reduce CO_2 emissions. In this review, the design and selection of a complete industrial scale biobutanol production plant, using microalgae as the feedstock, have been proposed. Advances in bioprocess technologies for biobutanol production via fermentation and biobutanol recovery methods are described. In addition, comparative analyses of biobutanol versus petroleum diesel and biodiesel, and strategies for biobutanol cum lipid and methane gas manufacturing are also discussed.

1. Introduction

The wide-spread modernization with an ever-increasing population, the hallmark of 21st Century, continues to strain global energy supplies. As petroleum and fossil fuels steadily deplete with concomitant increase in prices, extensive efforts have been made by the scientific community to search for and/or develop alternative sources of energy. To this end, renewable energy in the form of biofuels is being pioneered to meet the energy demands of the present-day society [1]. The escalating problem of global warming, as an assured consequence of greenhouse gas emissions from fossil fuels/petroleum, is also a prime motivator for this progress.

The development of 3rd generation biofuels sourced from cultivation of microalgae is an encouraging prospect for exploiting renewable energy resources. This is primarily because of the associated characteristic advantages as compared to the earlier approaches of biofuel production from crops (1st generation biofuels) and plant waste (2nd generation biofuels) [2]. In contrast to 1st generation biofuels, clearly, microalgae have no competition with agricultural crops for resource and land space allocation. They possess the capability to grow and thrive in different territories as compared to the region-sensitive crops, with significantly swifter harvest cycles [3]. Compared to 2nd generation biofuel materials, the simple structural characteristics of microalgae make them less recalcitrant as a biomass feedstock with simple and benign processing and conversion technologies [4]. These advantages have allowed microalgae to be a prime source for biomethanol, bioethanol, biohydrogen and bio-syngas production [5].

Biobutanol is conceived as a suitable replacement for conventional fuels due to an ensemble of advantages. As a biofuel, it is more effective than biomethanol or bioethanol, because of its higher energy density and its molecular similarity to gasoline. This indicates that it is more readily usable for the in-place fuel engines: either as a blend with diesel

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(good inter-solubility), or on its own without any modification [6]. The fact that it possess almost half the heat of vapourization of that of ethanol [7], indicates its superiority over either ethanol or methanol, when it comes to engine initiation at subzero temperatures [3]. Furthermore, it has a lower vapour pressure and lower volatility which facilitates easier storage and transport, and subsequently makes it less prone to problems like pipeline rupture, cavitation and vapour lock [8,9]. Besides being used as a fuel, it also has applications as a solvent in food and pharmaceutical industries [10]. Undoubtedly, it possesses a superior application range over other biofuels [11].

In spite of the admirable benefits associated with microalgae and the notable significance of biobutanol, only a limited number of reported work is focused on the fermentation of microalgae biomass to butanol, and most of the reported studies are only confined to the laboratory stage [12–15] with no commercial scale production reflections or appraisals to create a value proposition. To date, there seems to be lacunae in terms of reasonable research targeting the use of microalgae in large-scale biobutanol production. Current major commercial production of butanol is restricted to the oil and gas industry, as crude oil is processed to manufacture various petroleum products. The process initiates with propene which undergoes hydroformylation reaction, and is reduced with hydrogen to produce a mixture of butanol and iso-butanol [16]. The end product typically has a gas stream containing unreacted propylene, propane, unreacted carbon monoxide and hydrogen, resulting in economic deprivation and environmental pollution [17]. The recent instability over oil prices, the growing concerns over the greenhouse gases, and the depleting oil reserves, all have contributed to the relevance and recent surge in biofuel production [9].

Exploiting the scope of renewable energy exploration and environmental protection, several past studies have reviewed the importance of microalgae as a crucial source of biofuel production [4,5,18,19], and the processes and economics of biobutanol production [6,9]. Contextually, the current study will lay a comprehensive review of the potential application of microalgae cultivation to propose an industrialized biobutanol production process. A general approach to the proposed process flow diagram (PFD) of a biobutanol production plant would be classified into two distinct platforms: the first focuses on biomass production from microalgae; while the second focuses on biobutanol production via anaerobic fermentation (Fig. 1).

2. Microalgae feedstock production

2.1. Selection of microalgae with implemented bioengineering

The most desirable choice of microalgae would be the one with an inherently high starch content and productivity. This would be supplemented by nutrient limitation in order to encourage enhanced carbohydrate concentrations in the microalgae. Carbohydrates, which exist mostly as cellulose within the cell wall or as starch in plastids, are the major products of photosynthesis and carbon fixation in the Calvin cycle [20,21]. The absence of any lignin or hemicellulose contents lends precedent to its ease of fermentation in sizeable quantities. The composition of carbohydrates varies between different species of microalgae. Ideally, a strain with the highest concentration of convertible sugars from the cell wall and starch storage, should be selected. Past analyses have shown a list of microalgae strains with their carbohydrate contents [20]. Particularly, *Tetraselmis subcordiformis*, *Chlorella* sp. AE10, *Chlorella vulgaris*, *Chlorella reinhardtii* and *Scenedesmus obliquus* have demonstrated a high carbohydrate content (Table 1) [22–38]. Of these, *C. vulgaris* is notable for its high growth rate and high temperature tolerance in vitro [39]. Indeed, *C. vulgaris* has been used as a satisfactory feedstock for testing biobutanol fermentation in the laboratory [12,15].

2.2. Algae cultivation

Algae can be grown either in open pond systems or closed photobioreactors (PBRs). Prospects and limitations of algae cultivation systems have been evaluated and widely reported [40,41]. Open ponds are generally cheaper and easier to construct, but are susceptible to contaminations that can affect the quantity and quality of biomass and biochemical production capacity. Quality control can be ensured through the use of closed PBR systems. PBR systems minimize contamination with a better control of flow hydrodynamics and bioreaction conditions depending on the type. Hence, an efficient PBR cultivation process for maximizing algae biomass cultivation is dependent on successful implementation in an industrial-scale process, one that is energy efficient and economically feasible. Vertical column and flat-plate PBRs represent the two most viable options for algae cultivation [40–43]. While the vertical column PBRs are inherently batch systems, the flat-plate PBRs are continuous systems with easier construction methodology and lower operating costs. For instance, a combination of

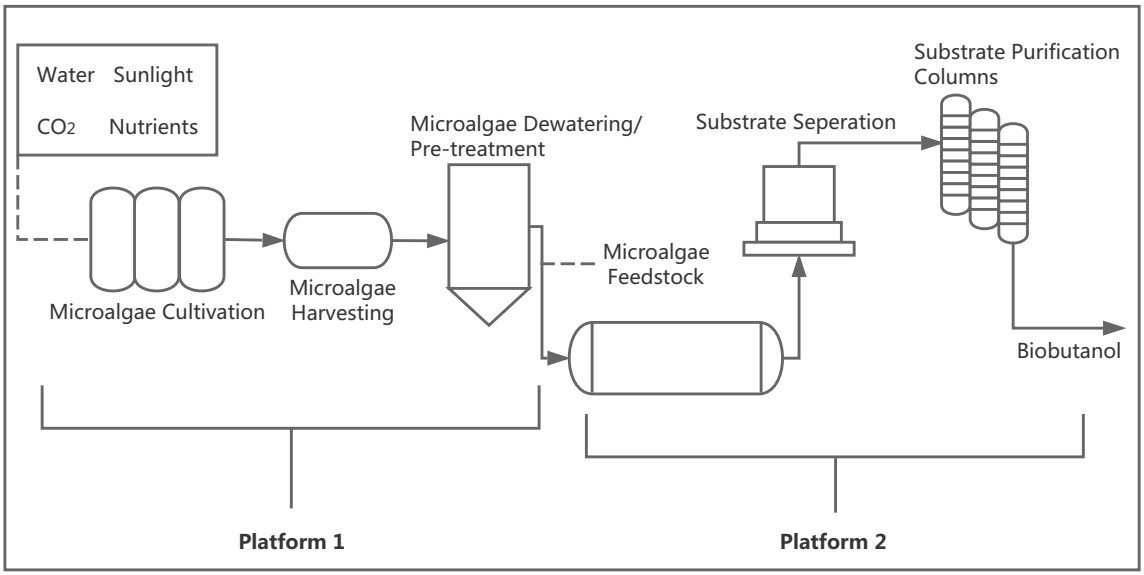

Fig. 1. Simplified process flow diagram of the proposed biobutanol production plant.

Table 1
Microalgae carbohydrate content, the top 11 were adapted from Chen et al. [20], the rest from Cheng et al. [27].

Microalgae species	Carbohydrate/starch content (%)	Carbohydrate productivity ($g\,L^{-1}\,d^{-1}$)	References
Chlorella vulgaris IAM C-534	37.0 (starch)	N/A	[36]
C. vulgaris CCAP 211/11B	55.0	0.021	[35]
C. vulgaris P12	41.0 (starch)	0.199 (starch)	[38]
C. vulgaris	55.0 (starch)	0.112	[22,34]
Chlamydomonas reinhardtii UTEX 90	60.0	0.304	[36]
C. reinhardtii IAM C-238	55.0 (starch)	N/A	[33]
Chlorococum sp.	32.5	N/A	[32]
Chlorococcum sp. TISTR8583	26.0 (starch)	N/A	[31]
S. acutiformis TISTR 8495	16.4 (starch)	N/A	[30]
S. obliquus CNW-N	51.8	0.383	[29]
Tetraselmis sp. CS-362	26.0	N/A	[28]
Scenedesmus obliquus CNW-N	52.9	0.468	[37]
Neochloris oleoabundans HK-129	41.3	0.047	[26]
C. zofingiensis	66.9	0.407	[25]
T. subcordiformis	62.1 (starch)	0.62 (starch)	[24]
C. fusca	49.0 (starch)	0.38 (starch)	[23]
Chlorella sp. AE10	77.6	0.421	[27]
	60.3 (starch)	0.311 (starch)	

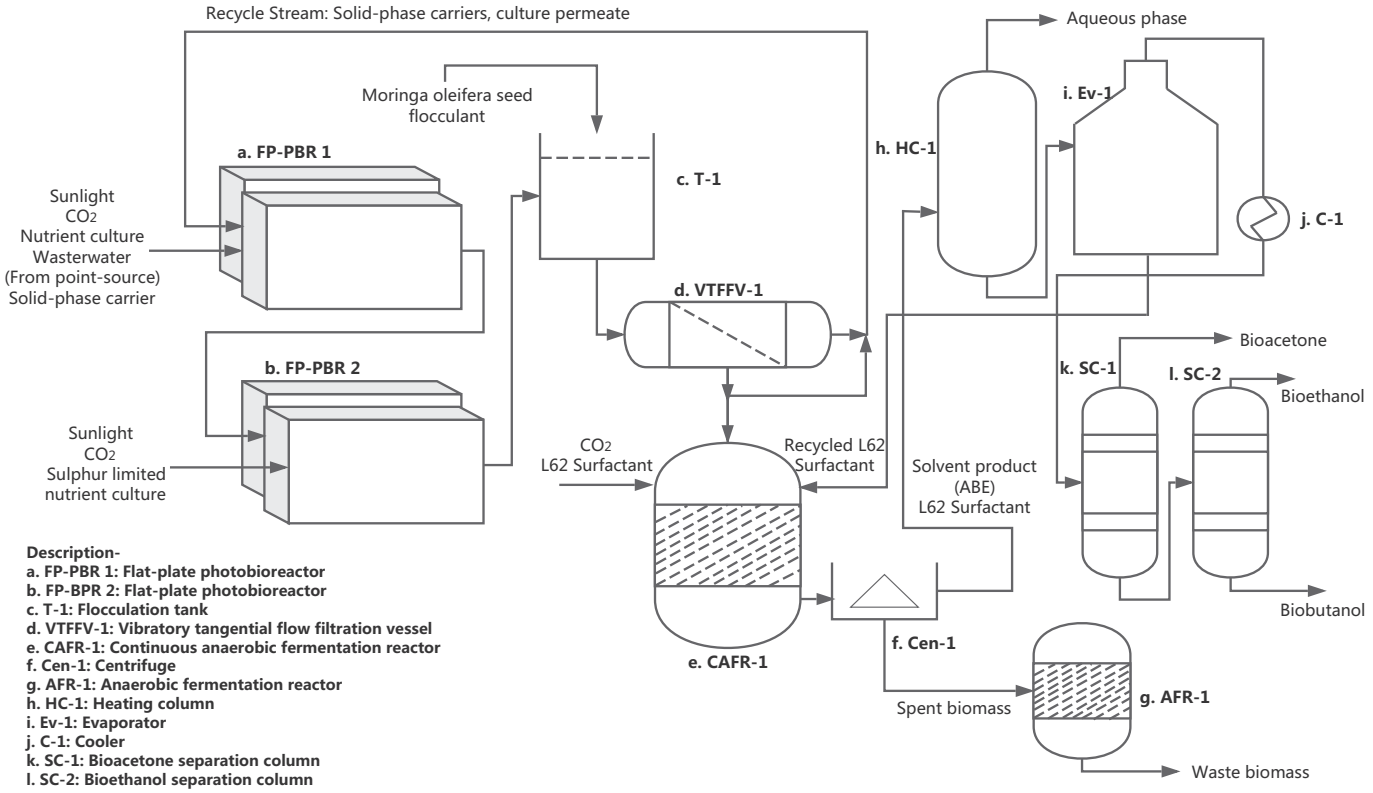

Fig. 2. Detailed process flow diagram of the proposed biobutanol production plant.

several flat-plate PBR trains would be easier to monitor as compared to hundreds of vertical column PBRs, which have to be individually maintained, cleaned and harvested. Thus, a practical, large-scale application would envisage flat-plate PBRs as the most suitable option of microalgal cultivation, with the inclusion of novel suspended-solid phase PBR (ssPBR) [43]. While CO_2 supply may be sourced from a supplier such as flue gas, if the chosen location is in close proximity to an industrial plant [44]; the culture may be derived from wastewater which would be ideally sourced from poultry farms due to their high nutrient contents [14,45]. With the implementation of nutrient limitation, it is possible to further focus the development of carbohydrates in the algal cells. Particularly, an earlier work has highlighted sulphur limitation as being crucial for a two-step microalgal cultivation process [46]. Here, the cells would initially grow in the culture before being transferred to the sulphur limited culture, to induce carbohydrate concentrations in the stationary phase. Thus, a two-stage cultivation process may be implemented where the first stage would pose as the growth phase of microalgae (Fig. 2a), and the second stage (stationary phase), where the culture is sulphur restricted, would encourage carbohydrate accumulation (Fig. 2b). The system would be aligned from north to south to maximize illumination during the day time [47].

2.3. Harvesting and dewatering method

A combination of harvesting/dewatering methods has been repeatedly cited in literature as the best way to obtain algal biomass [48–50]. This targets the process of separating water from the algal biomass culture, which can be a tedious and energy-consuming process.

Prior to this, it is important to also thicken the microalgae by converting it into a slurry. Common methods of dewatering include flocculation, centrifugation, filtration and sedimentation. While lab-scale implementation favors centrifugation, large-scale production would often necessitate flocculation and sedimentation [51]. Flocculation has the potential to be a cost-effective method of microalgae dewatering. Identifying an inexpensive, replenishable, non-toxic and effective flocculent is crucial for effective microalgae dewatering. To that end, an earlier study [52] has focused on the use of natural flocculent: *Moringa oleifera* seeds, that contain active bio-coagulative compound for the *Chlorella* sp. Flocculation efficiency in this case was shown to be very high (> 97%), as compared to the chemical coagulant: aluminium sulphate (> 37%). The concentration of biomass flocculated was 1.65-fold greater at 20 mg L^{-1}, but decreased to 1.59-fold for the 30 mg L^{-1} dosage.

Numerous studies in literature describe centrifugation as the simplest algae dewatering method. However, large-scale implementation of the process often results in high energy and capital costs [48,51]. Compared to other membrane filtration methods, dynamic filtration (vibratory membrane tangential flow filtration, TFF) has several advantages over conventional static filtration (tangential cross-flow membrane filtration or dead-end filtration). The most prominent being that high flow rates can be applied to cause reduced fouling resistance, resulting in longer operation periods with less membrane clean-up. The shear rate causes fewer algae to deposit on the membrane, allowing a higher transmembrane flux. It has been reported that dynamic filtration resulted in superior membrane permeability (50 L h^{-1} m^{-2} bar^{-1} for *Phaeodactylum tricornutum*) compared to conventional static filtration (10 L h^{-1} m^{-2} bar^{-1}, 500% improvement) with no fouling observed in some cases [48]. Thus the harvesting and dewatering method could involve a two-stage process. The first stage is to flocculate the microalgal culture using a natural flocculent like *M. oleifera* (Fig. 2c), followed by a second stage adaptation of dynamic filtration such as TFF (Fig. 2d) [48]. Filtration membrane will incorporate novel polytetrafluoroethylene (PTFE) material from Lee et al. [49] which was used specifically for *C. vulgaris*. The high flocculation efficiency, availability, and non-toxic nature of *M. oleifera*, along with the use of PTFE in a vibratory TFF, demonstrate the suitability of highly effective dewatering methods based on the respective studies. It should be noted, that the use of *M. oleifera* for industrial-scale biobutanol production, could generate considerable market potential for the plant, attributing to its pharmaceutical applications [52]. The ssPBR carriers from the culture would be removed from the filtration retentate, and recirculated back into the cultivation system for recycling. This is also the case for the filtration permeate.

3. Biobutanol production

3.1. Bacterium selection with implemented bioengineering

The bacterium *Clostridium acetobutylicum* remains a favorable option to help enhance biobutanol production due to its increased competence, as evident by the existing studies [9,12,13,15,53,54]. While the employment of other bacterial species has been reported earlier for biobutanol production (for instance *Escherichia coli* [54,55]), their adaptation in industrial plants is not supplemented with encouraging performance data. Thus, there is a pressing need for further incremental and novel research in future, that would lend feasibility to the use of other bacterial species in order to successfully produce and scale up biobutanol production.

The bacterium involved in the fermentation process is inhibited by its butanol tolerance. The cell membrane of the bacterium acquires more fluid-like characteristics due to the hydrophobic nature of butanol. This consequentially leads to membrane rupture and thus, kills the butanol-producing bacterium. Bacteria tend to react to solvent stress by influencing the cell membrane fluidity through the production of heat shock proteins or by the action of solvent efflux pumps [56]. This could have negative economic implications on the biobutanol production process, as it limits the amount of biobutanol produced. Although this necessitates replenishment, the slow anaerobic growth rate of *C. acetobutylicum* further complicates this problem. Typically, a wild strain of *C. acetobutylicum* demonstrates a butanol tolerance of 1.5% (v/v) [57].

Non-ionic surfactants were used by Dhamole et al. [58], which essentially isolate the manufactured butanol solvent from *Clostridium pasteurianum*, thereby protecting it without inhibiting further solvent production. These surfactants (Triton X 114, L64, L62LF, L61 and L62) were mixed with the fermentation broth and tested for biocompatibility. A 3% volume fraction of L64 showed reduced butanol yields, whereas Triton X 114 (3% volume fraction) inhibited solvent production. Optimized L62 yielded a 225% increased butanol yield of 106.8 g L^{-1}. Separation was achieved by evaporation of butanol from the surfactant-rich phase at a temperature range of 120–130 °C [59]. The results showed the effectiveness of L62, both as a solvent production booster as well as a good extractant for solvent separation, via the technique of cloud point extraction.

3.2. Biobutanol production via ABE fermentation

Biobutanol is produced by the acetone-butanol-ethanol (ABE) fermentation process, where microalgal biomass would be used as a substrate and is introduced to *C. acetobutylicum* bacterium for anaerobic digestion [54]. The bacterium first produces butyric and acetic acids through a process called acidogenesis, followed by solventogenesis, where butanol, acetone and ethanol are synthesized [6]. The primary microalgal constituent involved in this process is carbohydrate/starch, which is stored either as cellulose or in plastids. Production of acetate and butyrate reduces the culture pH, which induces a metabolic reaction leading to solvent production. It is a simpler process compared to the bioethanol production, as *C. acetobutylicum* is saccharolytic. This indicates an absence of a pre-treatment (such as starch liquefaction) step for either amylase or the saccharification action. The inherently simpler microalgal physiological structure ensures minimal pre-treatment measures, which in turn poses economic advantages over other feedstock forms. This leads to an acetone, butanol and ethanol ratio of 3:6:1 [9,53]. However, to our knowledge, microalgae as a substrate for large-scale ABE fermentation has not been exhaustively investigated.

There are primarily three methods to undertake fermentation: batch fermentation, fed-batch fermentation and continuous fermentation (Fig. 2e). Ranjan and Moholkar [9] reviewed the economics of these three fermentations, and suggested that, for large-scale production, continuous fermentation represents the ideal option as it forgoes the down-time costs intrinsically prevalent in batch and fed-batch fermentation processes, which have mostly to do with broth incubation and harvesting. Li et al. [60] compared the performance of the three processes and found that continuous fermentation showed considerable butanol yield and the highest butanol productivity at a pH of 4.5. When a pH of 4.5 was maintained, it was found that butanol solvent production was improved as compared to the uncontrolled pH condition. Time is always an integral factor for the accommodation of bacterium transition between the acidogenesis and solventogenesis phases. Hence, it was recommended that a low dilution rate should be used for the continuous fermentation to reduce this time period.

Processing remains the prime differentiator between the methods. Batch and fed-batch methods are more productive with greater retention times. This allows microorganisms to adapt and utilize the substrate provided, resulting in complete biotransformation of the substrate, while the environmental parameters like temperature and pressure are maintained. For continuous fermentation, reactor is initiated at batch mode where cells are allowed to grow until the growth reaches the exponential phase. During the exponential phase, the reactor is continuously fed with culture, and solvent is removed from the

vessel simultaneously, to maintain the liquid volume [61]. In this system, continuous input of substrate is proportioned with in situ removal of solvent products [9].

In addition to the introduction of L62 surfactant as described above, efforts have been made to alleviate the problems previously countered during continuous fermentation. Earlier studies have demonstrated improved butanol productivity using clay bricks to immobilize the fermentation bacteria as they are not accumulated along with the broth for downstream butanol separation [62,63]. Qureshi et al. [62] highlighted that the maximum solvent productivity obtained was 2.5-fold better, as compared to the typical cell immobilization methods. The bacterium was also shown to have no loss in solvent production after repeated batch cycles, or after 500 h of continuous operation, indicating that this application is favorable for such type of unit operation. It is to be noted that the clay bricks are typical construction bricks, which are cheap and considerably easy to procure.

3.3. Biobutanol separation and purification

Biobutanol separation remains an expensive process as ABE broth is largely dilute, with an offering yield of $< 20\ g\ L^{-1}$ [60]. This is additionally compounded by high energy usage, low volumetric productivity, and high substrate costs. Since butanol toxicity may adversely affect the fermentation bacterium, ideal recovery methods must balance solvent concentration with bacterium butanol tolerance. It has been suggested that solvent recovery be performed in situ from the ABE broth, which helps to preserve the fermentation bacterium [6,64]. Accordingly, this has been the cheaper and simpler way to sustain production (the other being genetic modification to improve the bacterium butanol tolerance [65]). Qureshi et al. [66] conducted a comparative study on energy consumption for recovery of butanol from the fermentation broth between liquid-liquid extraction (LLE) and pervaporation. It was concluded that adsorption using silicate was the most energy efficient ($8.16\ MJ\ kg^{-1}$) method.

3.3.1. Liquid-liquid extraction

LLE involves continuous contact between the fermentation broth and a hydro-immiscible ABE extractant, leaving all other broth components alone. This is typically accomplished employing a rotator disc column, and is followed by extractant distillation to acquire the ABE solvents [9]. Selection of extractant is a major process factor for consideration, and the extractant should have a high selectivity for ABE (with an emphasis on butanol). This method is advantageous for butanol compared to ethanol, as the former is more hydrophobic and less miscible with water, allowing for easier separation.

Extractive fermentation integrates solvent extraction within the bioreactor. This addresses the problem of butanol toxicity as the solvent is removed in situ, which improves bacterium productivity; while fermentation occurs alongside extraction. The extractant should demonstrate high distribution coefficient, high separation factor, low solubility in aqueous solution, low viscosity, large interfacial tension, high stability, low economic cost and nontoxicity towards bacterium/environment [67].

Oleyl alcohol was shown to have high extraction productivity by Davison and Thompson [68] using fluidized-packed bed reactor with immobilized *C. acetobutylicum*. Product yield increased to 50–90% and organic-aqueous ratio of butanol productivity increased from 1 to $4.18\ g\ L^{-1}\ h^{-1}$. This was the maximum productivity with a butanol distribution coefficient of 3. Ranjan and Moholkar [9] also showed that oleyl alcohol had the highest distribution coefficient at 3.21–4. In another study, a mixture of oleyl alcohol and decanol as extractants in a two-stage fermentation process, with immobilized cells and using liquid-liquid extraction integration, yielded $2.5\ g\ L^{-1}\ h^{-1}$ [69]. Furthermore, Groot et al. [70] examined 36 different chemicals in order to determine their compatibilities in liquid-liquid extraction of biobutanol; castor oil, oleic acid and isopropyl myristate, highlighted for their availability, high distribution coefficient, high selectivity and nontoxicity. It was pointed out that proper selection of extractant is necessary to avoid undesired precipitation and emulsion formation.

Another notable, cheaper and nontoxic extractant is the biodiesel. Although it has a lower distribution ratio than oleyl alcohol (0.91 vs. 2.8), it is advantageous since it manifests a suitable fuel blend that may be readily used for consumption. This completely bypasses the extractant-solvent separation procedure, and thus is more economical. As reported by a previous work, the butanol recovery was $16.7\ g\ L^{-1}$ [71].

Ionic liquids (IL) have shown considerable promise in butanol recovery, demonstrating high selectivity and distribution coefficients, with 1-hexyl-3-methylimidazolium tris (pentafluoroethyl) trifluorophosphate reporting the best performance overall [15,72]. ILs are characterized by their negligible vapour pressures, organic solubility, ability to prevent solvent loss, and a high degree of modification, allowing for greater selectivity for butanol. Their extremely low volatility ensures a simple and comparatively easier extractant-solvent separation process. While ILs have suitable characteristics that aid in butanol recovery, there seems to be limited data in literature on various ILs and their biocompatibilities to the fermentation bacteria. Moreover, although it is better than the traditional extractant, hexane [13,15], there is a concern regarding the high toxicity of IL towards the bacterium. Especially due to the fact that certain IL in fact inhibited metabolic processes that are crucial for the successful carrying out of the fermentation operation [73].

It should be noted, however, that with an increase in the production scale, LLE becomes less feasible due to enhanced extractant requirements, longer organic mass transfer rates, and process inhibition due to precipitation/emulsion. Intensive research efforts are underway targeting the LLE method to overcome the aforementioned, notable technical challenges.

3.3.2. Pervaporation

Pervaporation utilizes a membrane that is selectively permeable to ABE solvents from the fermentation broth. The ABE solvents get solubilized into the membrane following diffusion and are desorbed on the permeate side [9]. Separation occurs due to a chemical potential gradient and ABE is recovered by condensation in vapour form. Efficiency depends on the membrane permeability as well as its selectivity and in general, both are inversely affected by the other (i.e. improved permeability causes reduced selectivity, and vice-versa) [6].

Attributing to the aforementioned reasons, much interest has been focused on composite materials containing polymers and inorganic fillers (ceramic, zeolites such as silicalites, and so on), and possessing hydrophobic properties that are ideal for butanol separation. The economic aspect demands a balance between the low-cost, easily fabricated polymers, and the expensive, high strength, and highly stable inorganic materials. Notable examples of composite materials include variants of polydimethylsiloxane (PDMS): PDMS/ceramic [74], PDMS/silicate [75], PDMS/zeolites [76], PDMS/PAN [77], and pervatech PDMS [78], which exhibit high permeability, laudable selectivity, and ease of molding towards tubular/flat arrangement (Table 2). In addition, a hollow fiber module filled with ceramic-supported PDMS composite membranes, possessing outstandingly high and stable total flux and separation factors, was demonstrated by Liu et al. [79] to be a competitive candidate for the pervaporation application targeting practical biobutanol production. Further comparative analysis determined that poly (1-trimethylsilyl-1-propyne) (PTMSP) showed the best performance from a permeability and selectivity perspective [56]. Another option is the use of polyether block amide (PEBA), where novel implementation of carbon nanotubes (CNT) caused a 26% increase in butanol productivity and a 18% yield enhancement, as compared to PEBA without CNT (Table 2) [80]. CNT allowed improved permeability without compromising on selectivity, including an enhanced operational longevity aided by its mechanical strength [80]. Silicalite membranes, coated with 0.3% silicone-rubber, and using a porous tube for a

Table 2
Comparison between pervaporation membrane composite materials.

Membrane	Total permeate flux ($kg\,m^{-2}\,h^{-1}$)	Separation factor, BuOH	References
PDMS/ceramic	0.338–0.847	5–27	[74]
PDMS/silicalite	5.0–11.2	25–42	[75]
PDMS/ZSM-5 zeolite	1.5	45	[76]
PDMS/PAN	1.4	22.0	[105]
Pervatech PDMS	2.5	33.5	[78]
PDMS/hollow fiber	1.0	22.2	[79]
PEBA + CNTs	0.147	18	[80]
PEBA	0.297	11.57	[85]
MCM-41/PEBA	0.5	25.0	[84]
$Zn(BDC)(TED)_{0.5}$/PEBA	1.3	20.5	[83]
PEBA/hollow fiber	4.2	21.5	[82]
Silicone-rubber-coated silicalite	0.038	465	[81]

1% butanol solution pervaporation separation process, exhibited exceptional butanol concentration in permeate due to very high selectivity. However, the process suffered from a drawback in the form of reduced permeate flux as compared to other membrane types [81]. By dip-coating the ceramic hollow fiber with PEBA polymer solution, Li et al. [82] obtained a high pervaporation flux of $4.2\,g\,m^{-2}\,h^{-1}$. This may be because of the low transport resistance in the thin ceramic hollow fiber, which is higher than other polymer membranes commonly utilized for butanol recovery (Table 2) [80,83–85].

Past studies have demonstrated that energy consumption is inversely proportional to butanol concentration in the feed and butanol separation factor [67]. Thus, ensuring that both the latter conditions are met, will help mitigate energy costs pertaining to butanol recovery. Negishi et al. [81] quantified the energy consumption for using silicone-rubber coated silicalite membrane, and found that $4.3\,MJ\,kg^{-1}$ allowed for high butanol concentration in permeate.

In summary, pervaporation is one of the most prominent and viable methods for butanol recovery. It is characterized by high separation efficiency (differential transport through membrane pores), high energy efficiency (energy input for permeate evaporation), complete biocompatibility (no interaction with the fermentation bacterium) and logistical flexibility (good scale-up, and simplicity of operation and control) [86,87]. Fouling should be taken into consideration as it may damage and inhibit membrane performance. This is even more so, during prolonged operational periods.

Based on above analyses, a process flow diagram for biobutanol separation and purification is proposed. Cloud point extraction utilizes an evaporator column to separate the butanol (Fig. 2i). Once fermented broth is removed from the reactor, it is necessary to remove the surfactant L62. Separation of the surfactant and biobutanol would first require centrifugation (necessary to separate fermentation solids from liquids; following which, the fermented solids can be recycled into microalgae cultivation system, Fig. 2f), followed by heating in heating columns, which will incubate the mixture to induce phase separation (Fig. 2h). Two phases will form with the surfactant rich-phase at the bottom, and the aqueous phase at the top. Here, the solvent products from fermentation, will be concentrated in the surfactant rich-phase. This surfactant rich phase is then processed in an evaporator where the bottom product will mostly consist of the surfactant L62, which will then be recycled back into the fermentation reactor (Fig. 2e,i). The top product would then consist of solvents which would be processed through the separation trains (Fig. 2j–l), before finally reaching the storage facility/consumer.

Apart from using microalgal biomass as the feedstock, the advantage of the proposed concept lies in 2 modifications during continuous fermentation to manage potential solvent toxicity. The first being the immobilization of the solvent producing bacteria *C. acetobutylicum* on simple clay bricks to resolve the issue of bacterium replenishment every time it is undesirably flowed together with the fermented broth downstream for solvent product separation and purification. The second modification is the addition of non-cationic L62 surfactant into the fermentation broth to isolate the butanol product from the fermentation bacterium. This can effectively alleviate the problem solvent toxicity that inhibits microbial activity and leads to low production yields. Another advantage is that the process of separation and purification is made easier as cloud-point separation is utilized to effectively separate the L62 surfactant from the biobutanol product. This surfactant can then be recycled back into the fermentation reactor for reuse.

4. Comparative analyses of biobutanol, petroleum diesel and biodiesel

4.1. Biobutanol versus petroleum diesel

As biobutanol production is still in its infancy, direct life cycle assessment (LCA) between biobutanol and petroleum is mostly unfeasible, as the overall cost of production (even by current theoretical predictions), remains significantly higher. Despite this, biobutanol has shown promise in being much less energy intensive and is associated with less greenhouse gas emissions, both in terms of production and actual usage (Table 3). The production of petroleum diesel through fractional distillation of crude oil requires a process temperature between 200 °C and 350 °C at atmospheric pressure [88], as compared to anaerobic fermentation, which has an operational temperature range between 25 °C and 45 °C [89]. It has been reported that combustion of one gallon of petroleum diesel produces 22.38 pounds of CO_2 [90]. As stated previously, biobutanol characteristics have made it a potential candidate for fuel blending. Rakopoulos et al. [91] analyzed the fuel blend of butanol-diesel at different ratios for emissions in an engine speed of 2000 rpm for 3 separate pressure loads. The authors reported consistent improvement in emission reductions with increasing butanol blend ratios, implying that butanol-diesel blends (and to that extent, biobutanol) pose to be more eco-friendly.

4.2. Biobutanol versus biodiesel

In Table 4, comparative characteristics between biobutanol and biodiesel are summarized. Although the key factor or difference for the production of the two biofuels lies in their inherent biochemistry; for all purposes, the specifications for microalgae cultivation are the same. Biobutanol relies more on carbohydrates, while lipids take that role for biodiesel. As previously mentioned, fermentation is less energy intensive at 25–45 °C [89], while biodiesel transesterification with alkali catalyst requires 60 °C [92]. The fact that butanol contains more oxygen content, helps in lowering the soot emissions. Its higher heat of evaporation also causes fewer emissions of nitrogen oxide based gases. The average cost of biodiesel from palm oil was reported to be $ 0.66/L [92]. For microalgae biodiesel to be competitive in the market, it has to bear a cost either the same as or less than that of petro diesel. This is

Table 3
Comparison between biobutanol and petroleum diesel.

	Biobutanol	Petroleum diesel
Base process material	Microalgae biomass	Crude oil
Butanol production process	Anaerobic fermentation	Fractional distillation
By-product	Acetone, ethanol, methane gas (fermentation of spent biomass)	–
Cost	Higher	Lower
CO_2 emissions	Lower	Higher
Energy consumption	Lower	Higher

Table 4
Comparison between biobutanol and biodiesel.

	Biobutanol	Biodiesel
Base process material	Microalgae biomass (carbohydrates)	Microalgae biomass (lipids, requires extraction process) + methanol
Reaction additives	Non-ionic surfactant	Acid/alkali catalyst
Chemical process	Anaerobic fermentation	Transesterification
By-product	Bioacetone, bioethanol, methane gas (fermentation of spent biomass)	Glycerol (separated by phase separation)
CO_2 emissions	Lower (on combustion)	Higher (on combustion)
Energy consumption	Lower (separation of L62 surfactant + separation of ABE components)	Higher (biomass pretreatment + lipid extraction + transesterification process)

often compounded by the exorbitant production cost associated with biomass pre-treatment (for instance, acid or alkali pretreatment, heat application, etc.), followed by lipid extraction processes (liquid–liquid extraction (LLE), oil press, supercritical fluid extraction (SFE) and ultrasound) [4].

Lardon et al. [93] conducted an LCA analysis of 1 kg biodiesel production with both nitrogen deficient cultures, and normal cultures using *C. vulgaris*. A deficit in energy balance (−2.6 MJ) was reported when the biodiesel was produced with normal cultures and dried biomass was obtained. Nitrogen deficient cultures induced greater lipid production and thus, resulted in greater biodiesel production, which consequently, accounted for a positive energy balance (12 MJ). Drying had the highest energy input (81.8 MJ), which was attributed to the initial deficit. This step could be overlooked in favor of wet lipid extraction (i.e., solvent extraction). However, in spite of the fact that this step increased the energy requirement for lipid extraction, the amount was within the acceptable limit as compared to the same in case of drying.

To conclude, biobutanol separation as proposed in Fig. 2, shows promise in being a cheaper and more energy efficient alternative in comparison to biodiesel production.

5. Life cycle assessment of microalgal biomass versus other feedstocks for biobutanol production

Appreciating and understanding the economics of microalgae cultivation is important to determine the feasibility of the biobutanol production plant. Thus, Table 5 provides a comparison with other feedstock the cost of biomass production, overall energy input and carbon emission assessment. This study is adapted from Clarens et al. [94] which reports LCA study of biomass production based on a functional unit of 317 GJ of biomass-derived energy. The impact areas covered were energy consumption (MJ), water usage (m^3), greenhouse gas emissions (kg CO_2 equiv), land (ha) and eutrophication potential (kg PO_4^{3-} equiv).

From the table, it can be deduced that algae biomass has an advantage in terms of efficient land utilization. Sensitivity analysis indicates that the primary drivers for high energy and greenhouse gas (GHG) emissions are high heating value (HHV) (i.e., the energy content embodied by algal biomass and released during combustion), CO_2 production and use, and fertilizer requirements.

Carbon dioxide is a crucial component in photosynthesis as it participates in cell growth. Through biosequestration, CO_2 can be used to drive microalgae cultivation for biomass development. This gives microalgae one of their distinct advantages over current carbon capture and storage (CCS) technology. Microalgae comprise of 50% carbon, meaning approximately 1.83 tons of CO_2 is required to produce 1 ton microalgae. The cultivation system provides a method for constant supply of CO_2 into the culture. The estimation costs for CO_2 supply is significant, ranging between 8% to 27% [95] thus ensuring maximum carbon supply goes a long way in mitigating the cost. Mitigation through the use of flue gas as a carbon source is recommended in the literature [44,96]. Most of these mitigation strategies suggest the placement of microalgae cultivation plants within the vicinity of coal-fired power plants to allow easy procurement of CO_2 gas and reduce the life-cycle burden of algae biomass production. This synergy can effectively reduce the toxicity of gas emissions from the coal plant and reduce the cost of CO_2 procurement.

As CO_2 would be supplied to the culture stream as a gas, it is very important to minimize the escape of CO_2; hence, open race-way ponds are at a disadvantage due to the short CO_2 retention time. Carbon fixation is dependent not only on the feed flowrate of CO_2 gas, but the effective CO_2 mass transfer efficiency within the culture. This may be improved through effective mixing in conformance with the system geometry. It should be noted that for tubular PBRs, typical diameters are 5 cm for small-scale and 10–20 cm for middle-scale. Larger capacities require larger diameters and lengths, which may result in non-uniform CO_2 mass transfer. It was suggested by Tababa et al. [97] that a bubble column PBR design presented some distinct advantages over tubular PBRs in that it saves land area, reduces energy consumption and has greater adaptability to light conditions. Manipulation of gas hold-up and bubble size could help improve turbulence and mass transfer of CO_2 within the algal culture.

Fertilizer requirements greatly affect energy consumption and GHG emissions. The assessment is based on nutrient requirement for algal growth as opposed to fertilizer application for biomass crops. The excessive quantities of NPK fertilizers used in growing crop biomass feedstocks and the concomitant effects on the soil and water bodies are prohibitive. This study discusses the work reported by Ellis et al. [45] using municipal wastewater to supplement and mitigate nutrient for algae cultivation. Wastewater sources tend to exhibit varying concentrations of nitrogen and phosphorus depending on the nature of the source. Generally, agricultural sources have the highest nutrient concentration followed by municipal wastes which usually contains more heavy metals. Industrial wastewater contains heavy pollutants and fewer nutrients. Ellis et al. [45] demonstrated that the use of algae from the Logan City Wastewater Lagoon system obtained promising results for ABE fermentation of *Clostridium saccharoperbutylacetonicum* N1-4 in a batch fermentation process (2.74 g L^{-1}). The ABE yields increased with the addition of carbon source and enzymes, showing 160%

Table 5
Life cycle burdens of production of one functional unit of energy (317 GJ) from respective feedstock.
Adapted from Clarens et al. [94].

	Land (ha)	Energy (MJ) × 10^4	GHG (kg CO_2 equiv) × 10^4	Water (m^3) × 10^4	Eutrophication (kg PO_4^{2-} equiv)
Algae	0.40 ± 0.50	30.00 ± 6.6	1.80 ± 0.58	12.00 ± 2.40	3.30 ± 0.86
Corn	1.30 ± 0.30	3.80 ± 0.35	−2.60 ± 0.09	0.82 ± 0.19	26.00 ± 5.40
Canola	2.00 ± 0.20	7.00 ± 0.83	−1.60 ± 0.10	1.00 ± 0.14	28.00 ± 5.80
Switchgrass	1.70 ± 0.40	2.90 ± 0.27	−2.40 ± 0.18	0.57 ± 0.21	6.10 ± 1.70

increase (7.27 g L^{-1}) and 250% (9.74 g L^{-1}) increase, respectively. Emphasis was given to the importance of substrate pre-treatment as it improved the bio-availability of enzyme-substrate reactions. Acid pre-treatment involved substrate digestion with sulphuric acid at 90 °C and agitating for 30 min, while alkaline pre-treatment used sodium hydroxide at the same temperature and duration. An economic analysis of the wastewater treatment plant demonstrated that a theoretical yield of 33 tons of algae biomass per day was possible.

Wilson et al. [98] estimated that the cost of CO_2 capture for algae production was unlikely to be lower than ~$ 400/ton biomass even in the most optimistic scenario, and concluded that the overall economics of CO_2 capture and recycle depended strongly on the value of the algal biomass produced. Other arguments also point out that wastewater should be seen as a partial measure to meet the nutrient demands for microalgae cultivation and not the main source, as economics indicate that even optimistic estimates would only be able to accommodate 3% of US annual energy consumption if wastewater is fully utilized [13]. These concerns suggest that further efforts should be invested to improve the value of algae culturing, aiming to make the energy production larger than the energy requirement [99].

6. Combinations of biobutanol, lipid and gas production from microalgae

6.1. Sole biobutanol production versus biobutanol cum lipid production

As is the case with a petroleum refinery, utilizing virtually every component of the microalgae biomass for processing into usable either end or byproducts, represents an advance in bio refinery productivity. Lipids are a crucial component in the production process of biodiesel. Hence, recycling of the consumed biomass is typically encouraged in order to utilize the constituent lipids. Chisti [92] reported that the lipid content in *Chlorella* sp. is in the range of 28–32%. In a large-scale bio refinery, substantial volumes of lipids would be produced in the algal biomass which would ultimately be wasted, unless it is extracted. Hence, this would necessitate an entirely new process segment dedicated to the extraction microalgal lipids. Considering the option of lipid extraction prior to fermentation leads to the undesired issue of probable microalgal cell wall disruption. This is in order to access the lipids stored inside the cell, which induces pre-treatment costs. In addition, lipid extraction could adversely affect the fermentation efficiency. Chemical pre-treatment methods like extremely low acid (ELA), or mechanical pre-treatment methods like ultrasonification, are highly energy intensive for cell wall disruption. This may necessitate additional procurement of equipment and materials to achieve the desired outcomes. Hence, an entire evasion of this step may prove to be cost effective, by carrying out lipid extraction after ABE fermentation.

The lipid extraction process would be conducted following the centrifugation process (Fig. 2f), where the ABE solvent is separated from the consumed biomass. This may also act as the starting point for lipid extraction. By now, the ABE fermentation process would have provided the saccharolytic treatment necessary to break down the cell walls and other polysaccharides, making it easier to access the lipids in the broth. Solvent extraction by employing solvent systems, such as methanol/chloroform, hexane, hexane/alcohols, has often been the most popular route in lab-scale investigations. A typical and frequently reported solvent system is methanol-chloroform [100]. Application of solvent would normally be on dried algal biomass (necessitating an energy-intensive drying process). Hence, the impractical usage of large-scale solvent systems, and energy loads due to drying, suggest that a wet method (involving water) of lipid extraction is more preferable in order to extract the lipids. Indeed, this has been confirmed earlier in a biodiesel processing study [93]. A novel approach for lipid extraction was proposed by Yang et al. [101] using ethanol at room temperature and normal atmospheric pressure. A high lipid extraction yield of 33.04% dry weight was achieved through gentle stirring, and addition of 5 mL solvent per gram of the wet biomass for 37 min. The results showed similarities with the classic Bligh-Dyer method [101].

Overall, addition of lipid production process for the biobutanol plant would require a low-cost, energy efficient and low emission, which is practical for scale-up considerations. This process is best carried out following centrifugation of ABE fermentation broth, as biobutanol production should have the foremost priority.

6.2. Isolated biobutanol versus biobutanol cum methane gas production

Anaerobic digestion of the exhausted microalgae biomass may be carried out in order to produce methane gas. Methane gas is primarily used as a fuel source due to its high heat of combustion. Synergy between waste water plants and consumed algae biomass represents a potentially efficient process for biological wastewater treatment and fuel production. It is possible to further boost methane gas yield by addition of alternative biomass in the form of waste paper. This was reported in an earlier work [102], where a near two-fold increase (from 573 $mL^{-1}\,d^{-1}$ to 1170 $mL^{-1}\,d^{-1}$) could be observed. A techno economic analysis conducted by Harun et al. [103] focused on the utilization of consumed algal biomass in a biodiesel production plant. The objective was to produce methane gas as a fuel source to power the preceding biodiesel production process. It was found that sufficient power was produced by the methane gas to accommodate raceway pond energy requirements with a surplus (biodiesel production costs was improved by 33% as a result). In addition, PBRs showed a deficit in energy consumption due to more extensive usage of electrical equipments.

In the context of the biobutanol production plant, the methane gas produced subsequently, can either be sold off directly, or used to mitigate the energy consumption in microalgae cultivation and product purification/separation. As already shown in Fig. 2, an additional anaerobic fermentation reactor is necessary to produce the methane gas (Fig. 2g). This would naturally incur enhanced cost, energy consumption and carbon emissions (due to the production of CO_2 as a by-product). However, considering the potential volume of methane gas to be produced on large-scale basis, this energy consumption could immediately be recovered and even sustained, as has been theoretically predicted in an earlier work [104]. CO_2 produced from the fermenter can be recycled into the microalgae cultivation system or the continuous anaerobic fermentation reactor (Fig. 2a,b,e). Residual biomass from this second fermentation process can then be used for fertilizer production.

To summarize, implementing methane gas production process for the biobutanol plant, shows promise in reducing butanol production costs, carbon emissions and energy consumption.

7. Conclusion

The current review has analyzed a biobutanol manufacturing process with a focus on large scale algal cultivation, including an economic and life cycle assessment of the entire process. *C. vulgaris* remains the preferred choice for the microalgae to be cultivated due to an extensive past repertoire of literature and research evidences. Studies on algal harvesting and dewatering methods suggested the amalgamation of a flocculation – filtration process, whereby *M. oleifera* seeds could be used as flocculents, due to their superior flocculation efficiency. The filtration process should incorporate the use of a vibratory membrane along with a novel polytetrafluoroethylene (PTFE) membrane material. Continuous fermentation has emerged as an effective technology for microbial biobutanol production from biomass feedstocks. However, the technology is challenged with the problem of solvent toxicity that inhibits efficient production of biobutanol. The present study incorporates two modifications to address the problem of solvent toxicity: immobilization of the solvent producing bacteria, *C. acetobutylicum*, on simple clay bricks, and the addition of non-cationic L62 surfactant into

the fermentation broth. Furthermore, cloud point separation is utilized to effectively separate the L62 surfactant from the biobutanol product. This surfactant is then recycled back into the fermentation reactor for reuse without affecting efficacy. The current review also considered viable, additional manufacturing of either methane gas or lipids, that can be fed along with either algal biomass, and/or supplementary discarded biomass.

In conclusion, the merging of algal cultivation technology along with biobutanol production process shows plenty of promise, especially in the context of rejuvenating the biofuel industry. This is mainly due to efficacy of the combinatorial approach to counter the problems faced by inadequate fossil fuel resources, in addition to the detrimental effects of CO_2 emissions on an already sensitive environment. Detailed, in-depth analyses of both these processes could lead to improved comprehension, as to how productivity yield may be optimized for large-scale or even industrial grade implementation.

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Competing interests, statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Authors' contributions

The original idea was suggested by DM and ZX, and the concept of this study was developed in discussion with all authors. YT and JK drafted the article. LL and PS contributed to critical revision of the article for important intellectual content. All authors read and approved the final manuscript.

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